Comparison of in vivo transfection efficiency was conducted by injecting gelatin nanospheres containing the LacZ plasmid (1 mg total DNA), DNA-Lipofectamine complex (1 mg total DNA), naked plasmid DNA (1 mg), or 5×1010 viral particles of AAV-LacZ suspended in 20 ml into the exposed tibialis anterior muscle bundles of six-week old BALB/c mice. One and three weeks later, the muscle was isolated, homogenized, and the reporter gene expression determined by an assay kit (Galacto-Light') supplied by Tropix (Bedford, Mass.). Injection of gelatin nanospheres containing 1 mg of the LacZ gene into the tibialis muscle bundle of mice produced β-gal expression for at least 21 days (FIG. **22**). The level for an equivalent dose of naked DNA was 10–30 fold lower at day 7, and declined to background level by day 21. In contrast to their relative performance in vitro, the Lipofectamine complexes were not as efficient as the nanospheres. The expression level at day 7 was even lower than that of naked DNA. By gross observation, there was acute inflammatory response in the muscle tissue treated by the Lipofectamine complexes, which might account for the poor result. The AAV vector was the most efficient, eliciting a β-gal expression of 50–100 times higher than that of the nanospheres at day 7, and the level increased 6–12 fold further at day 21, probably due to viral replication.

Nanospheres synthesized by salt-induced complex coacervation of cDNA and polycations such as gelatin and chitosan are efficient gene delivery vehicles. DNA-nanospheres in the size range of 200–750 nm could transfect a variety of cell lines. Although the transfection efficiency of the nanospheres was typically lower than that of Lipofectamine and calcium phosphate controls in cell culture, the β-gal expression in muscle of BALB/c mice was higher and more sustained than that achieve by naked DNA and Lipofectamine complexes. This gene delivery system has several attractive features: 1) ligands can be conjugated to the nanosphere to stimulate receptor-mediated endocytosis; 2) lysosomolytic agents can be incorporated to reduce degradation of the DNA in the endosomal and lysosomal compartments; 3) other bioactive agents or multiple plasmids can be co-encapsulated; 4) bioavailability of the DNA can be improved because of protection from serum nuclease degradation by the matrix; 5) the nanosphere is stable in plasma electrolytes, and can be lyophilized for storage without loss of bioactivity.

We claim:

1. A composition comprising solid nanospheres of less than 3 μm for gene delivery to cells, comprising a polymeric cation and a polyanion, wherein the polyanion consists of nucleic acids, wherein the polymeric cation is a carbohydrate.

2. The composition of claim **1** wherein the polymeric cation is chitosan.

3. The composition of claim **1** wherein said nanospheres comprises greater than 5% (w/w) nucleic acids.

4. The composition of claim **1** wherein said nanospheres comprises greater than 20% (w/w) nucleic acids.

5. The composition of claim **1** wherein said nucleic acids comprise a gene of 2–20 kb.

6. The composition of claim **1** wherein the nanospheres are between about 200 and 300 nm.

7. The composition of claim **1** wherein the nanospheres are less than 2 μm.

8. The composition of claim **1** wherein the nanospheres are less than 1 μm.

9. The composition of claim **1** wherein the nanospheres are less than 151 nm.

10. A method of forming solid nanospheres for gene delivery to specific target cells, comprising the step of:

forming nanospheres of less than 3 μm by coacervation of nucleic acids and a polymeric cation which is a carbohydrate.

11. The method of claim **10** wherein the coacervation is performed in the presence of sodium sulfate.

12. The method of claim **10** wherein the polymeric cation is chitosan.

13. The method of claim **10** wherein the polymeric cation is present at a concentration of about 0.01–7% in the step of coacervation.

14. The method of claim **10** wherein the nucleic acids are present in a concentration of 1 ng/ml to 500 μg/ml in the step of coacervation.

15. The method of claim **11** wherein the concentration of sodium sulfate is between about 5 and 100 mM in the step of coacervation.

16. The method of claim **10** wherein the nanospheres are between about 200 and 300 nm.

17. The method of claim **10** wherein the nanospheres are less than 2 μm.

18. The method of claim **10** wherein the nanospheres are less than 1 μm.

19. The method of claim **10** wherein the nanospheres are less than 151 nm.

20. A method for introducing genes into cells, comprising the steps of:

incubating (a) cells to be transfected, in vitro with (b) solid nanospheres of less than 3 μm comprising polymeric cationic molecules and nucleic acid molecules, whereby the cells are transfected with the nucleic acid molecules, wherein the polymeric cationic molecules are carbohydrates.

21. The method of claim **20** wherein the polymeric cationic molecules are chitosan.

22. The method of claim **20** wherein the nucleic acid is DNA.

23. The method of claim **20** wherein the nucleic acid is RNA.

24. The method of claim **20** wherein the nanospheres are between about 200 and 300 nm.

25. The method of claim **20** wherein the nanospheres are less than 2 μm.

26. The method of claim **20** wherein the nanospheres are less than 1μm.

27. The method of claim **20** wherein the nanospheres are less than 151 nm.

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